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Susceptibilities of Anaerobic Bacteria to Cefoperazone and Other Antibiotics

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Two hundred fifty clinical isolates of anaerobic bacteria were tested for susceptibility to cefoperazone, cefamandole, cefoxitin, carbenicillin, clindamycin, and chloramphenicol. Anaerobic gram-positive cocci were susceptible to all of the antibiotics tested. Clindamycin was the most active agent against *Bacteroides* species, followed by chloramphenicol and then cefoxitin. Cefoperazone was less active than cefoxitin and equal in activity to carbenicillin. Cefamandole was the least active antibiotic against *Bacteroides*. *B. fragilis*, *B. distasonis*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. ovatus* were more resistant to the antibiotics than *B. melaninogenicus*, *B. oralis*, or *B. bivius*. Clindamycin was the most active agent against *Clostridium* species, followed by chloramphenicol; the three cephalosporins and carbenicillin were about equal in activity. Clindamycin was the most active antibiotic against *Fusobacterium* species, followed by chloramphenicol, carbenicillin, and cefoperazone (which were about equally active) and then cefamandole.

Cefoperazone is a cephalosporin analog of piperacillin sodium that is active against gram-positive cocci and gram-negative bacilli including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella-Enterobacter* species, *Proteus* species (indole-negative and -positive strains), and *Pseudomonas* species (1, 2). This study was undertaken to determine the activity of cefoperazone and other selected antibiotics (cefamandole, cefoxitin, carbenicillin, chloramphenicol, and clindamycin) against anaerobic bacteria.

MATERIALS AND METHODS

Organisms. Two hundred fifty clinical isolates of anaerobic bacteria were studied. Included were 28 strains of anaerobic gram-positive cocci (18 *Peptococcus* sp. and 10 *Peptostreptococcus* sp.); 55 strains of *Bacteroides fragilis*; 21 strains of *B. distasonis*; 20 strains of *B. vulgatus*; 20 strains of *B. thetaiotaomicron*; 13 strains of *B. melaninogenicus*; 13 strains of *B. ovatus*; 12 strains of *B. oralis*; 8 strains of *B. bivius*; 26 strains of assorted *Bacteroides* species (6 *B. corrodens*, 5 *B. capillosus*, 1 *B. asaccharolyticus*, and 14 strains of *Bacteroides* that could not be identified as to species); 28 strains of *Clostridium* species (12 *C. perfringens*, 2 *C. ramosum*, 2 *C. sporogenes*, 2 *C. sartagoformum*, 1 *C. subterminale*, 1 *C. glycolicum*, 1 *C. putrefaciens*, 1 *C. bifermentans*, 1 *C. ghoni*, 1 *C. difficile*, 1 *C. innocuum*, and 3 unidentified strains of *Clostridium*); and 6 strains of *Fusobacterium* species (1 *F. prausnitzii*, 2 *F. naviforme*, 1 *F. plauti*, 1 *F. nucleatum*, and 1 *F. necrogenes*).

Antibiotics. The antibiotics tested were supplied as: cefoperazone, Pfizer Pharmaceuticals, New York, N.Y.; cefamandole, Eli Lilly and Co., Indianapolis, Ind.; cefoxitin, Merck Sharp and Dohme, Inc., West Point, Pa.; carbenicillin, Roerig, New York, N.Y.; chloramphenicol, Parke-Davis, Detroit, Mich.; and clindamycin, The Upjohn Co., Kalamazoo, Mich.

In vitro susceptibility tests. The minimal inhibitory concentrations (MICs) were determined by an agar dilution method by the method of Sutter et al. (4). The antibiotics were diluted in twofold steps in water. One milliliter of each dilution of antibiotic was added to 9 ml of Wilkens-Chalgren agar (Difco Laboratories, Detroit, Mich.), which contains hemin and vitamin K_1, to obtain final antibiotic concentrations of 0.1 to 100 μg/ml. Bacteria were grown for 24 h in brain heart infusion broth, supplemented with hemin (5 μg/ml) and vitamin K_1 (0.5 μg/ml). Each culture was diluted with brain heart infusion supplemented broth to the turbidity of one-half the no. 1 McFarland standard and inoculated onto the surface of freshly prepared plates with the replicating device of Steers et al. (3). The replicator delivered approximately 3×10^5 colony-forming units of each strain in brain heart infusion broth. The MIC was considered to be the lowest concentration of antibiotic that allowed growth of no more than one colony after 48 h of anaerobic incubation at 37°C in a GasPak system (BBL Microbiology Systems, Cockeysville, Md.).

Controls included each time were aerobic incubation to rule out contamination with aerobes and anaerobic incubation without antibiotics. Three standard organisms were also run as controls each time: *C. perfringens* ATCC 13124, *B. fragilis* ATCC 25285, and *B. thetaiotaomicron* ATCC 29741.

RESULTS

As shown in Table 1, anaerobic gram-positive cocci were susceptible to all of the antibiotics studied. Ninety percent of the strains were in-

TABLE 1. *Comparison of cefoperazone, cefamandole, cefoxitin, carbenicillin, clindamycin, and chloramphenicol*

Microorganism (no. of strains)	Drug	MIC (μg/ml)			
		Range	For 50% of strains	For 75% of strains	For 90% of strains
Anaerobic gram-positive cocci (28)	Cefoperazone	<0.2–25	0.4	1.6	1.6
	Cefamandole	<0.2–12.5	0.2	1.6	6.3
	Cefoxitin	<0.2–25	0.2	1.6	6.3
	Carbenicillin	<0.2–25	0.4	0.8	6.3
	Clindamycin	<0.2–6.3	0.2	0.8	1.6
	Chloramphenicol	<0.2–6.3	1.6	3.1	6.3
B. fragilis, B. distasonis, and *B. vulgatus* (96)	Cefoperazone	0.4–>100	50	50	100
	Cefamandole	<0.2–>100	50	>100	>100
	Cefoxitin	0.2–50	6.3	25	50
	Carbenicillin	0.2–>100	50	100	100
	Clindamycin	<0.2–6.3	0.2	0.4	0.8
	Chloramphenicol	<0.2–12.5	6.3	6.3	6.3
B. thetaiotaomicron and *B. ovatus* (33)	Cefoperazone	<0.2–>100	50	100	100
	Cefamandole	1.6–>100	100	>100	>100
	Cefoxitin	1.6–100	25	50	100
	Carbenicillin	6.3–100	50	50	100
	Clindamycin	<0.2–6.3	0.8	3.1	3.1
	Chloramphenicol	1.6–12.5	6.3	6.3	6.3
B. melaninogenicus (13)	Cefoperazone	<0.2–100	0.4	1.6	100
	Cefamandole	<0.2–>100	3.1	50	>100
	Cefoxitin	<0.2–50	0.2	12.5	50
	Carbenicillin	0.2–50	1.6	6.3	50
	Clindamycin	<0.2–0.8	<0.2	<0.2	0.8
	Chloramphenicol	<0.2–6.3	0.4	3.1	3.1
B. oralis (12)	Cefoperazone	<0.2–100	6.3	50	50
	Cefamandole	0.2–100	12.5	50	50
	Cefoxitin	0.4–12.5	3.1	12.5	12.5
	Carbenicillin	0.2–>100	3.1	100	100
	Clindamycin	<0.2–0.4	<0.2	0.2	0.2
	Chloramphenicol	0.4–6.3	3.1	3.1	6.3
B. bivius (8)	Cefoperazone	0.2–3.1	0.8	1.6	3.1
	Cefamandole	<0.2–50	0.4	1.6	50
	Cefoxitin	<0.2–12.5	<0.2	0.8	12.5
	Carbenicillin	0.4–6.3	0.4	3.1	6.3
	Clindamycin	<0.2–0.2	<0.2	0.2	0.2
	Chloramphenicol	0.4–12.5	1.6	1.6	12.5
Bacteroides sp. (26)	Cefoperazone	<0.2–50	0.4	6.3	50
	Cefamandole	<0.2–100	6.3	25	50
	Cefoxitin	<0.2–25	3.1	12.5	12.5
	Carbenicillin	<0.2–50	1.6	12.5	25
	Clindamycin	<0.2–3.1	<0.2	0.4	1.6
	Chloramphenicol	<0.2–6.3	1.6	3.1	6.3
Clostridium sp. (28)	Cefoperazone	0.2–>100	3.1	6.3	>100
	Cefamandole	<0.2–>100	1.6	12.5	>100
	Cefoxitin	<0.2–>100	1.6	12.5	>100
	Carbenicillin	<0.2–>100	1.6	6.3	>100
	Clindamycin	<0.2–6.3	0.2	1.6	6.3
	Chloramphenicol	<0.2–12.5	3.1	6.3	6.3
Fusobacterium sp. (6)	Cefoperazone	<0.2–12.5	0.8	3.1	12.5
	Cefamandole	<0.2–25	0.4	12.5	25
	Cefoxitin	<0.2–50	3.1	12.5	50
	Carbenicillin	<0.2–6.3	1.6	6.3	6.3
	Clindamycin	<0.2–0.8	<0.2	0.2	0.8
	Chloramphenicol	0.8–6.3	0.8	3.1	6.3

hibited by 6.3 μg or less of each antibiotic per ml, and all strains were inhibited by 25 μg or less of each antibiotic per ml. There were only minor differences in susceptibility patterns between the 18 peptococci and the 10 peptostreptococci.

As the susceptibility patterns of *B. fragilis*, *B. distasonis*, and *B. vulgatus* were almost identical, these results were pooled in Table 1. Similarly, as the susceptibility patterns of *B. thetaiotaomicron* and *B. ovatus* were almost identical, these results were pooled. In general, clindamycin was the most active antibiotic against *Bacteroides* species, followed by chloramphenicol and then cefoxitin. Cefoperazone was less active than cefoxitin and equal in activity to carbenicillin. Cefamandole was the least active of the agents against *Bacteroides* species. *B. fragilis*, *B. distasonis*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. ovatus* were more resistant to the antibiotics than *B. melaninogenicus*, *B. oralis*, *B. bivius*, and the 26 other *Bacteroides* species (*B. corrodens*, *B. capillosus*, *B. asaccharolyticus*, and unidentified strains). Ninety percent or more of all of the different *Bacteroides* species were inhibited by 3.1 μg of clindamycin and 12.5 μg of chloramphenicol per ml. The *B. oralis* and *B. bivius* strains were much more susceptible to clindamycin than the others; 90% were inhibited by 0.2 μg/ml.

Cefoxitin inhibited 90% of most of the species of *Bacteroides* at concentrations of 12.5 to 50 μg/ml (the exception was *B. thetaiotaomicron*, which required 100 μg/ml). *B. bivius* strains were the most susceptible to cefoxitin; 75% were inhibited by 0.8 μg/ml, as compared with the other species, which required at least 12.5 μg/ml.

Cefoperazone and carbenicillin at 50 to 100 μg/ml were required to inhibit 75% of strains of *B. fragilis*, *B. distasonis*, *B. vulgatus*, *B. thetaiotaomicron*, *B. ovatus*, and *B. oralis*. In contrast, only 1.6 to 6.3 μg of cefoperazone or carbenicillin per ml was required to inhibit 75% of strains of *B. melaninogenicus* and *B. bivius*. The other *Bacteroides* species (*B. corrodens*, *B. capillosus*, *B. asaccharolyticus*, and unidentified strains) were midway in susceptibility between these two groups of species.

At least 100 μg of cefamandole per ml was required to inhibit 75% of each of the species of *Bacteroides* studied with the exception of *B. melaninogenicus*, *B. oralis*, *B. bivius*, and the 26 *Bacteroides* species; 50, 50, 1.6, and 25 μg/ml, respectively, were required to inhibit 75% of these latter strains.

Clindamycin was the most active antibiotic against *Clostridium* species, with chloramphenicol the next most active. All strains were inhibited by 6.3 μg of clindamycin and 12.5 μg of chloramphenicol per ml. The three cephalosporins and carbenicillin were about equal in activity; 6.3 to 12.5 μg/ml inhibited 75% of the strains.

Clindamycin was the most active antibiotic against *Fusobacterium* species; all strains were inhibited by 0.8 μg/ml. Chloramphenicol and carbenicillin were equally active; all strains were inhibited by 6.3 μg/ml. Cefoperazone was only slightly less active than these agents (all strains were inhibited by 12.5 μg/ml) and was more active than cefamandole (all strains were inhibited by 25 μg/ml). Cefoxitin was the least active; 50 μg/ml was required to inhibit all strains.

DISCUSSION

Clindamycin and chloramphenicol were the most active of the six antibiotics tested. There were no anaerobes highly resistant to either of these two antibiotics. Cefoxitin was more active than cefoperazone and carbenicillin against *Bacteroides* species but less active against *Fusobacterium* species. Cefoperazone was generally equal in activity to carbenicillin against the anaerobes. Cefamandole was the least active of the antibiotics against *Bacteroides* species.

The results with cefoperazone agree with those of Neu et al. (2) who tested 23 strains of *Bacteroides* species with an inoculum of 10^5 on Mueller-Hinton agar. Fifty percent were inhibited by 50 μg/ml, and 90% were inhibited by 100 μg/ml. However these investigators did not separate the species of *Bacteroides*. Inclusion of varying numbers of different species could have a major effect on making the *Bacteroides* more or less resistant. For example, *B. bivius* are very susceptible to cefoperazone, and *B. fragilis* are much more resistant.

Jacobus et al. (N. V. Jacobus, S. L. Gorbach, M. Barza, and F. P. Tally, Program Abstr. 11th Int. Cong. Chemother.-19th Intersci. Conf. Antimicrob. Agents Chemother., 1979, abstr. no. 134) reported strains of *B. fragilis* to be much more susceptible to cefoperazone than was found in the present study. They reported a median MIC of 16 μg/ml for *B. fragilis* as compared with 50 μg/ml in the present study. Jacobus et al. also reported a cefoperazone median MIC of 2 μg or less per ml for the *B. bivius-B. disiens* group. This is comparable to the 0.8 μg/ml in the present study. Details of media and inoculum size were not given.

The results of this study indicate that clindamycin and chloramphenicol were much more active than the other antibiotics tested. Although cefoperazone was equal in activity to carbenicillin and more active than cefamandole, it was less active than cefoxitin. Clinical trials will be required to determine the role of cefo-

perazone in infections caused by anaerobic bacteria.

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